

UNITED STATES DEPARTMENT OF AGRICULTURE ANIMAL AND PLANT HEALTH INSPECTION SERVICE  <b>ANNUAL REPORT OF RESEARCH FACILITY</b> (TYPE OR PRINT)	1. CERTIFICATE NUMBER: 14-R-0035 CUSTOMER NUMBER: 130	FORM APPROVED OMB NO. 0579-0036
University Of Massachusetts Medical School 55 Lake Avenue North Worcester, MA 01655  Telephone: (508) -856-3151		

3. REPORTING FACILITY (List all locations where animals were housed or used in actual research, testing, or experimentation, or held for these purposes. Attach additional sheets if necessary)

FACILITY LOCATIONS (Sites) - See Attached Listing

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY (Attach additional sheets if necessary or use APHIS Form 7023A)

A. Animals Covered By The Animal Welfare Regulations	B. Number of animal being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes.	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals an for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.	E. Number of animals upon which teaching, experiments, research, surgery or tests were conducted involving accompanying pain or distress to the animals and for wh the use of appropriate anesthetic, analgesic, or tranquiliz drugs would have adversely affected the procedures, res or interpretation of the teaching, research, experiments, surgery, or tests. (An explanation of the procedures producing pain or distress in these animals and the reaso such drugs were not used must be attached to this report	F. TOTAL NUMBER OF ANIMALS  (COLUMNS C + D + E)
4. Dogs	0	0	26	0	26
5. Cats	0	0	1	0	1
6. Guinea Pigs	0	472	1281	681	2434
7. Hamsters	0	239	33	1350	1622
8. Rabbits	2	285	114	0	399
9. Non-human Primates	2	0	25	14	39
10. Sheep	0	0	0	0	0
11. Pigs	0	4	95	0	99
12. Other Farm Animals	0	0	0	0	0
13. Other Animals	0	0	0	0	0

ASSURANCE STATEMENTS

- Professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anesthetic, analgesic, and tranquilizing drugs, prior to, during, and following actual research, testing, surgery, or experimentation were followed by this research facility.
- Each principal investigator has considered alternatives to painful procedures.
- This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and an Institutional Animal Care and Use Committee (IACUC). A summary of all such exceptions is attached to this annual report. In addition to identifying the IACUC-approved exceptions, this summary includes a brief explanation of the exceptions, as well as the species and number of animals affected.
- The attending veterinarian for this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL  
 (Chief Executive Officer or Legally Responsible Institutional Official)

SIGNATURE OF OFFICIAL (Type or Print)

DATE SIGNED

(b)(6), (b)(7)(c)

*QaQ*

**Registration Number: 14-R-0035**

**Species: Guinea Pigs**

**Total numbers of guinea pigs in Cat E: 402 + 46 + 93 + 140 = 681**

1. Guinea Pigs: 402

Procedure

The MLD (minimum lethal dose) Test – Diphtheria Toxin is used to quantify the toxin activity of each batch of diphtheria toxin manufactured at MBL and intended for use in the production of diphtheria toxoid vaccines. It is used as one measure of toxin potency. The test uses three guinea pigs; each is given a different dose (the doses are 1, 3, and 5 ml) of a diluted diphtheria toxin preparation. The dilution of the toxin is based on an in vitro measurement of toxin antigenicity versus a standardized anti-serum to diphtheria toxin. Guinea pigs are observed twice daily for symptoms of diphtheria intoxication or death for up to five days after inoculation. The FDA requirement for diphtheria toxin intended for use in the manufacture of diphtheria toxoid vaccine is that the minimum volume of toxin needed to kill a guinea pig within 4 days of injection into the animal corresponds to a value of  $\geq 400$  MLD/ml of toxin. Each batch of diphtheria toxin intended for use in MBL vaccine manufacturing must meet this toxicity requirement.

Scientific justification why pain or distress could not be reduced or eliminated

Guinea pigs inoculated with a sufficient amount of toxin will exhibit symptoms of diphtheria toxicity or possibly death by the 96-hour endpoint of the assay. However, symptoms alone cannot be used as an endpoint to assess assay validity or toxin potency. Any use of pain-relieving drugs would interfere with the results of the assay.

Federal regulation required

The FDA requires that each lot of Diphtheria toxin pass the Minimum Lethal Dose criteria as described in the NIH Minimum Requirements: Section 1.3, 4<sup>th</sup> revision dated March 1, 1947 before it can be released for further manufacturing to produce vaccine.

2. Guinea Pigs: 46

Procedure

The Immunoglobulin Diphtheria Potency Test is used to measure the amount of antibody to diphtheria toxin (anti-toxin) in human immunoglobulin preparations. The FDA requires that any immunoglobulin released and distributed for human use have no less than 2 antitoxin units/ml. Guinea pigs are used in the test. The control is a known amount of a standard diphtheria toxin mixed with an FDA reference anti-toxin unit/ml; the test is a known amount of standard diphtheria toxin mixed with the immunoglobulin under test. Both mixtures are incubated for one to six hours so that the antibody can neutralize the

**Registration Number: 14-R-0035**

effects of the toxin. The mixtures are injected subcutaneously into two guinea pigs each. The two animals injected with the mixture of the standard diphtheria toxin and FDA reference anti-toxin must die within 96 hours. The two animals injected with the mixture of immunoglobulin product and a standard diphtheria toxin must survive longer than 96 hours indicating the presence of at least 2 anti-toxin units/ml. Each lot of vaccine manufactured by MBL must pass the Immunoglobulin Diphtheria Potency Test, as described in 21CFR 640.104, before it can be released for distribution.

Scientific justification why pain or distress could not be reduced or eliminated.

If there is not enough anti-toxin present to neutralize the toxin in the mixtures, the animals will die from the effects of diphtheria toxin. The test is designed so that the controls will die within 96 hours and the test animals will survive longer. Four to five days after injection of the immune globulin-toxin mixture all survivors are euthanized. Pain relieving drugs can not be used because they may interfere with the detection of diphtheria intoxication symptoms or with the time of death used as the endpoint in the assay.

Federal regulation required

The only test for diphtheria potency accepted by the FDA for immune globulin, intramuscular, intended for human use is the animal model described in NIH Minimum Requirements: Diphtheria Antitoxin, 2<sup>nd</sup> revision, Jan. 18, 1946 and 21CFR 640.104. Anti-diphtheria potency of immune globulin lots is most accurately measured by using guinea pigs to test the ability of the immune globulin to neutralize diphtheria toxin.

**3. Guinea Pigs: 93**Procedure

The Diphtheria Potency Test for Vaccines is performed on all lots of vaccine intended for use in humans. A group of eight guinea pigs is immunized subcutaneously with 0.5 mL of vaccine or 2.0 ml of its precursor toxoid. Four weeks after immunization blood is drawn intracardially from anesthetized animals. The sera from all animals are pooled and dilutions of the serum pool are incubated in-vitro with a known amount of diphtheria toxin. The ability of antibodies in the guinea pig serum pool to neutralize the toxin is measured by injecting the toxin: serum mixtures intraperitoneally into pairs from another group of guinea pigs. The potencies of the vaccine and its precursor toxoid are calculated from the highest serum pool dilution that protects guinea pigs from death. Each lot of vaccine manufactured by MBL must pass the potency requirement in order for it to be released for distribution.

Scientific justification why pain or distress could not be reduced or eliminated

NOV 30 2005

**Registration Number: 14-R-0035**

Guinea pigs inoculated with toxin: antiserum mixtures must be observed for symptoms of diphtheria toxicity or death at the 96-hour end point of the assay. Pain is minimized in any animals inoculated with the toxin-antiserum mixtures by allowing the test period to extend only long enough to observe the animals at the 96-hr time point required by the test, and by observing the animals for symptoms of toxicity or death twice daily during the test. Any use of pain-relieving drugs may interfere with the results of the assay.

Federal regulation required

The FDA requires that each lot of diphtheria vaccine pass the animal protection potency test described in the NIH Minimum Requirements: Section 3.3, 4<sup>th</sup> Revision dated March 1, 1947, NIH Minimum Requirements: Tetanus and Diphtheria Toxoids Combined Precipitated Adsorbed (For Adult Use), Section 3.2, dated August 25, 1953, and NIH Minimum Requirements: Tetanus and Diphtheria Toxoids Combined Precipitated Adsorbed (For Adult Use), Amendment No1, dated November 28, 1956 before the lot can be released for distribution.

4. Guinea Pigs: 140

Procedure

The MLD (minimum lethal dose) Test – Tetanus Toxin is used to quantify the toxin activity of each batch of tetanus toxin manufactured at MBL and intended for use in the production of tetanus toxoid vaccines. It is used as one measure of toxin potency. The test uses guinea pigs that are inoculated subcutaneously with diluted tetanus toxin. The inoculated animals are observed for 120 hours. The data are the time intervals between inoculation and death. From this time period the potency of each batch of tetanus toxin is calculated and expressed as the MLD. The dilution of the toxin used to inoculate each animal is based on an in vitro measurement of toxin antigenicity versus a standardized anti-serum to tetanus toxin. The data obtained from the test are used to show the potency of each batch of tetanus toxin produced and to show that the purity of the toxin meets the criteria established in the NIH (FDA) guidelines for tetanus toxoid vaccines

Provide scientific justification why pain or distress could not be reduced or eliminated.

Guinea pigs inoculated with a sufficient amount of toxin with exhibit symptoms of tetanus toxicity or death during the 120-hour time period of the assay. Symptoms alone, however, cannot be used as an endpoint to assess assay validity or toxin potency. The MBL product license for tetanus/diphtheria vaccine, contain an assay to measure toxin activity by its MLD in guinea pigs. The MLD is calculated according to the monogram published by J. Ipsen, Arch. Exptl. Pharmacol. 197:836-889 (1941). Thus, MLD is dependent on the time of death. The cited procedure did not use pain-relieving drugs. Any use of pain-relieving drugs would interfere with the results of the assay because 1)

NOV 30 2005

**Registration Number: 14-R-0035**

the test cited in the product license did not use them, and 2) drug use may alter the time-of-death and, thus, the MLD determination.

Federal regulation required

The test is performed according to the NIH Minimum Requirements for Tetanus Toxoid, 4<sup>th</sup> revision, dated December 15, 1952. It is a requirement of MBL's product license for the manufacture and distribution of tetanus/diphtheria vaccine. The tetanus potency test is required by 21CFR610.10. A review of the Code of Federal Regulations, revised April 1, 2004, indicates that the test is required for each lot of vaccine intended for human use. No alternative method is available.

**Species: Hamsters**

**Total Number in Cat E: 1350**

Procedure

*Clostridium difficile* is a gram positive anaerobic bacillus which is responsible for antibiotic-associated diarrhea diseases infecting up to 20% of individuals admitted to hospital. *Clostridium difficile* elaborates two toxins, Toxin A and Toxin B. Hamsters are challenged with *C. difficile* bacteria. Using this animal model we have developed a human monoclonal antibody directed against Toxin A that, in combination with polyclonal sera reactive to Toxin B, can protect 80-100% of hamsters from death associated with *Clostridium difficile*. The main goal of this study is to raise polyclonal serum to multiple fragments of the Toxin B molecule and test this serum in the hamster challenge model in order to inform our decision as to which antigen to choose for use in our anti-toxin B monoclonal antibodies.

Provide scientific justification why pain or distress could not be reduced or eliminated

The disease induced by these bacteria results in death of the hamster. Death is mediated by destruction of the animal intestine as well as systemic toxicity. We intend to monitor the animals at least two times a day to detect signs of disease such as diarrhea, inactivity, and weight loss. Animals exhibiting these features will be euthanized as to alleviate pain associated with the disease. Once overt symptoms appear in the animal, death usually occurs within 12 hours. As such, euthanasia is preferable to the administration of analgesics. We have designated all animals as Category E except those which are not given the disease (i.e., controls). These control animals are listed as Category C.

Federal regulation required

None.

NOV 30 2005

**Registration Number: 14-R-0035**

**Species: Nonhuman Primates**

**Total Number in Cat E: 14**

Procedure

These studies involve the Magnetic Resonance Imaging (MRI) of the conscious marmoset. In primates, body movement has been shown to obscure and sometimes even mimic changes in signals that correspond to neuronal activation. In MRI studies, there must be a device to immobilize the head and body of the animal without restricting the animals' vital functions. The overall intent is to facilitate the acquisition of neuroimages that will enhance our understanding of the neuronal circuitry involved in the stress response.

The stress caused by immobilization and noise from the MRI scanner in a fully conscious animal is a major concern. We therefore adapt animals to the imaging procedures (and measure basal levels of hormones and resting levels of autonomic activity) in order to isolate the stress-mediated changes in brain activity from those of interest.

Scientific justification why distress cannot be relieved

This non-invasive in vivo method of neuroimaging in awake animals is designed to change our current imagery of the brain from either a static entity (as seen with most neurochemical measurements) or a low activation of dynamic system in an anesthetized state (as seen with current MRI or PET measurements), to a real time 3-D physiologically functioning unit. Therefore, to accomplish this work in the non-anesthetized animal, and to stop body movement signals (as described above) we provide restraint in a specially made apparatus to minimize the stress to the animal.

Federal regulation required

None

NOV 30 2005

**Registration Number: 14-R-0035**

Sites for USDA Certificate No. 14-R-0035

1. University of Massachusetts Medical School

(b)(2)High, (b)(7)f

2.

(b)(7)f, (b)(2)High